

## PATENT COOPERATION TREATY

**PCT**

REC'D 24 SEP 2001  
WIPO PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>788190/98002</b>	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/116)
International application No. <b>PCT/US00/11400</b>	International filing date (day/month/year) <b>28 APRIL 2000</b>	Priority date (day/month/year) <b>29 APRIL 1999</b>
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 15/82 and US Cl.: 435/430, 430.1, +69		
Applicant <b>SALEM-TEIKYO UNIVERSITY</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

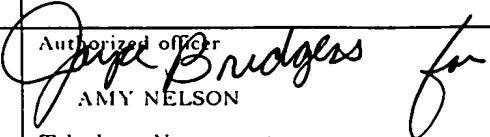
2. This REPORT consists of a total of **5** sheets.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of **0** sheets.

3. This report contains indications relating to the following items:

- I  Basis of the report
- II  Priority
- III  Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV  Lack of unity of invention
- V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement
- VI  Certain documents cited
- VII  Certain defects in the international application
- VIII  Certain observations on the international application

Date of submission of the demand <b>28 NOVEMBER 2000</b>	Date of completion of this report <b>31 AUGUST 2001</b>
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer  AMY NELSON
Facsimile No. (703) 305-8250	Telephone No. (703) 308-0196

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/11400

**I. Basis of the report****1. With regard to the elements of the international application:\*** the international application as originally filed the description:pages 1-40, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_ the claims:pages 41-52, as originally filed  
pages NONE, as amended (together with any statement) under Article 19  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_ the drawings:pages 1-14, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_ the sequence listing part of the description:pages NONE, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_**2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.**

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).  
 the language of publication of the international application (under Rule 48.3(b)).  
 the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

**3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:**

- contained in the international application in printed form.  
 filed together with the international application in computer readable form.  
 furnished subsequently to this Authority in written form.  
 furnished subsequently to this Authority in computer readable form.  
 The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
 The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

**4.  The amendments have resulted in the cancellation of:**

- the description, pages NONE  
 the claims, Nos. NONE  
 the drawings, sheets/fig. NONE

**5.  This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\***

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.  
PCT/US00/11400**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

the entire international application.

claims Nos. 17-33,54-128

because:

the said international application, or the said claim Nos. \_ relate to the following subject matter which does not require international preliminary examination (*specify*).

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_ are so unclear that no meaningful opinion could be formed (*specify*).

the claims, or said claims Nos. \_ are so inadequately supported by the description that no meaningful opinion could be formed.

no international search report has been established for said claims Nos. 17-33, 54-128

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the standard.

the computer readable form has not been furnished or does not comply with the standard.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/11400

**V. Reasons statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

<b>Novelty (N)</b>	Claims <u>39,41,42,50</u>	<b>YES</b>
	Claims <u>1-16,34-38,40,43-49,51-53</u>	<b>NO</b>
<b>Inventive Step (IS)</b>	Claims <u>NONE</u>	<b>YES</b>
	Claims <u>1-16,34-53</u>	<b>NO</b>
<b>Industrial Applicability (IA)</b>	Claims <u>1-16,34-53</u>	<b>YES</b>
	Claims <u>NONE</u>	<b>NO</b>

**2. citations and explanations (Rule 70.7)**

Claims 1-15 lack novelty under PCT Article 33(2) as being anticipated by Rogers (*In Vitro Cellular and Developmental Biology* 34: 75A, 1998).

Rogers teaches a method of transforming a plant cell, specifically a cell of *Typha latifolia*, by inoculation with *Agrobacterium* comprising a vector comprising the *gus* reporter gene (abstract). Although the reference does not specifically recite that the *Agrobacterium* is disarmed and that the reporter gene is operably linked to control sequences, such aspects of *Agrobacterium* transformation are so well known in the art that they were not specifically recited in the reference, and are considered to be inherent characteristics of the *Agrobacterium* disclosed by Rogers. Rogers also discloses transformation of *Typha latifolia* with a gene coding for specific pollutant decontamination activities (*i.e.* bioremediation). Hence, all of the claim limitations were previously disclosed by Rogers.

Claims 34-38,40,43-49,51-53 lack novelty under PCT Article 33(2) as being anticipated by Sarma *et al.* (*Plant Cell Reports* 17: 656-660, 1998).

Sarma teaches a method for plant regeneration from callus, specifically regeneration of plants of *Juncus acuminatus*, comprising shoot induction by treatment with benzyladenine (BA), followed by root induction by treatment with NAA, both under continuous illumination (abstract; p. 657, left-hand column). The regenerated plants were grown to maturity and produced viable seed (p. 660). Hence, all of the claim limitations have been disclosed by Sarma.

Claims 34-53 lack an inventive step under PCT Article 33(3) as being obvious over Sarma *et al.* (*Plant Cell Reports* 17: 656-660, 1998) in view of Fridborg (Biosis Database on STN Abstract No. 2+8966).

The teachings of Sarma are discussed above.

Sarma does not teach a method for regeneration of plants of *Juncus effusus*, *Carex lurida* and *Scirpus polyphyllus*. Also, Sarma does not teach said method wherein the starting cells are transgenic, nor wherein the shoot induction further comprises (Continued on Supplemental Sheet.)

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

International application No.

PCT/US00/11400

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):**

treatment with charcoal, citric acid or ascorbic acid.

Fridborg discloses that treatment with charcoal enhances differentiation in plants (abstract).

It would have been obvious to modify the invention of Sarma to substitute other plant species for *Juncus accuminatus*, such as *Juncus effusus*, *Carex lurida* or *Scirpus polyphyllus*. The claimed plants are closely related to *Juncus accuminatus*, being in the same genus or family, and one would have expected to achieve similar results with the related plant species. It further would have been obvious to regenerate from transgenic cells rather than from non-transgenic cells, because one of the primary goals for developing plant regeneration methods is for regeneration of transgenic plants. Moreover, it would have been obvious to modify the invention of Sarma to also treat plants during shoot induction with charcoal, given the teachings of Fridborg showing that it enhances differentiation. One would have had a reasonable expectation of success in view of the success of both Sarma and Fridborg.

**----- NEW CITATIONS -----**

NONE

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/11400

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 15/82

US CL :435/430, 430.1, 469

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/430, 430.1, 469

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, AGRICOLA, CAPLUS, BIOSIS, EMBASE, USPAT  
search terms: carex, juncus, typha, scirpus, gramineae, transformation, regeneration

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE BLOCK et al. The Cell Biology of Plant Transformation: Current State, Problems, Prospects and the Implications for Plant Breeding. Euphytica. 1993, Vol. 71, pages 1-14, see entire article.	1,7-15 ----
Y	WALDEN et al. Cell Culture, Transformation and Gene Technology. In: Plant Biochemistry and Molecular Biology. Edited by LEA et al. New York: John Wiley and Sons. 1999. pages 335-343, entire document.	2-6,16 ----
X	ROGERS et al. Gus Expression in Typha latifolia (Cattail) Cells Transformed with Agrobacterium. In Vitro Cellular and Developmental Biology. Abstract No. P-1130. March 1998, Vol. 34, No. 3, Part II, page 75A.	1,7-15 ---- 2-6, 16 1-16

 Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 SEPTEMBER 2000

Date of mailing of the international search report

22 SEP 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

AMY NELSON

Telephone No. (703) 308-0196

Facsimile No. (703) 305-3230

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/11400

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----	SARMA et al. Plant Regeneration and Multiplication of the Emergent Wetland Monocot Juncus accuminatus. Plant Cell Reports. 1998, Vol. 17, pages 656-660, see entire article.	34-38,40,43- 49,51-53 ----
Y		39,41,42,50
X ----	ROGERS et al. Shoot Regeneration and Plant Acclimitization of the Wetland Monocot Cattail (Typha latifolia). Plant Cell Reports. 1998, Vol. 18, pages 71-75, see entire article.	36-38,40,43- 49,51-53 ----
Y		39,41,42,50
X ----	SARMA et al. High Frequency Plant Regeneration from Callus Cultures of Typha angustifolia. In Vitro Cellular and Developmental Biology. Abstract No. P-1126, March 1998, Vol. 34, No. 3, Part II, page 74A.	34-38,40,43- 45,47-49,52,53 ----
Y		39,41,42,46,50,51
Y	Database BIOSIS on STN. JONES et al. 'The Role of Photosynthesis and Oxidative Reactions in Leaf Blackening of Protea-Neriifolia R. Br. Leaves'. Sci. Hortic. Abstract No. 330797. 1992, Vol. 50, No. 1-2, pages 137-145.	50
Y	Database BIOSIS on STN. FRIDBORG et al. 'The Effect of Activated Charcoal on Tissue Cultures Adsorption of Metabolites Inhibiting Morphogenesis'. Physiologia Plantarum. Abstract No. 248366. 1978, Vol. 43, No. 2, pages 104-106.	50



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> :	A1	(11) International Publication Number:	WO 00/66757
C12N 15/82		(43) International Publication Date:	9 November 2000 (09.11.00)

(21) International Application Number:	PCT/US00/11400	(81) Designated States:	AE, AG, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, DZ, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date:	28 April 2000 (28.04.00)		
(30) Priority Data:			
60/131,513	29 April 1999 (29.04.99)	US	
60/171,127	16 December 1999 (16.12.99)	US	
(71) Applicant (for all designated States except US):	SALEM-TEIKYO UNIVERSITY [US/US]; P.O. Box 500, 223 West Main Street, Salem, WV 26426-0500 (US).		
(72) Inventor; and			
(75) Inventor/Applicant (for US only):	ROGERS, Suzanne, D. [US/US]; 513 Stout Street, Bridgeport, WV 26330 (US).		
(74) Agents:	DORTENZO, Megan, D.; Steptoe & Johnson, P.O. Box 2190, Clarksburg, WV 26302-2190 (US) et al.		

(54) Title: CLONED AND ENGINEERED PLANTS AND METHODS OF USE FOR BIOREMEDIALTION

## (57) Abstract

The present invention recognizes that plant cells, particularly plant cells from freshwater monocot plants, can be transformed and regenerated, particularly to produce plants that have bioremediative capacities. The present invention includes methods for transforming and regenerating a plant, preferably a freshwater monocot plant cell such as the freshwater emergent wetland monocots *Carex*, *Scirpus*, *Juncus* or *Typha*. The present invention includes cells and populations of cells, including callus, plants and seeds, made by or derived from this method. The present invention also includes methods of bioremediation by exposing a plant of the present invention to an environment or sample that contains or is suspected of containing at least one contaminant that can be reduced by the plant.

## Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.